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AMV Reverse Transcriptase

Cat. No: XV0070P (300 U) [(10 U/µL)]

Cat. No: XV0071P (1000 U) [(10 U/µL)]

Cat. No: XV0071P-CS (1000 U) [(20 U/µL)]

Introduction

AMV Reverse Transcriptase, encoded by Avian Myeloblastosis Virus (AMLV) is an RNA-dependent DNA polymerase that synthesizes the complementary cDNA first strand from a single-stranded RNA template.

AMV Reverse Transcriptase (AMV RT) catalyzes the polymerization of DNA using template DNA, RNA or RNA: DNA hybrids. The enzyme possesses an intrinsic RNase H activity. AMV RT possesses multiple enzymatic activities including RNA- and DNA-directed DNA polymerase, DNA-RNA unwinding activity, a sequence-specific Mn2+-dependent endonuclease and ribonuclease H.

Source: Purified from E. coli strain harboring a plasmid that directs the synthesis of modified form of AMLV-RT.

Application

- RT PCR
- Synthesis of DNA
- RNA sequencing

Kit Contents

- AMV Reverse Transcriptase
- 5X Reaction Buffer

Storage

Upon receipt of the kit, immediately store the components at -20 °C.

Unit Definition:

One unit is the amount of enzyme required to catalyze the transfer of 1nmol of deoxynucleotide into acid-precipitable material in 10 minutes at 37°C, using poly(A) oligo dT as a template primer.

Quality Control

AMV RT is free of detectable RNase, and DNase (exo- and endonuclease) activities.

Purity: >90% as judged by SDS-polyacrylamide gels with blue staining.

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Protocol

We recommend preparing 2 mixes:

1. Mix 1: Add 2 µg of total RNA (or 50 – 500 ng of poly(A)-RNA) to reverse DNA primer. Use 0.5µg primer/µg RNA in a total volume of 11µl in water. Mix gently by vortex.
2. Mix 1: Incubate the mixture at 70°C for 5 minutes and chill on ice.
3. Mix 2: Mix the following in the tube:
    - 5 µl of 5X RT buffer
    - 2.5 µl of dNTPs mix (10mM each)
    - [Optional] 20-40 Units RNase inhibitor (not provided)
    - 2.5 µl 40 mM Sodium Pyrophosphate (prewarmed to 42°C)
    - 3 µl of AMV Reverse Transcriptase (10U/µl)
    - Nuclease-free water to final volume of 25 µl
4. Combine Mix 1 and Mix 2, vortex gently.
5. Incubate the reaction:

    Incubate for 60 minutes at 42°C for oligo(dT) primers or at 37°C for random hexamer primers.
6. Stop the reaction by heating at 70°C for 10 minutes. Chill on ice.
7. Use the mixture for the desired application.

PRODUCT USE LIMITATION This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.